L-Arginine Supplementation Enhances NOS2 Expression in Human Renal Cell Carcinoma

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Abstract

Renal cell carcinoma (RCC) is responsible for 80% of all adult cancers. Biochemical pathways such as the urea cycle play a major role in the proliferation of these tumors. Arginase (ASE) and inducible nitric oxide synthase (NOS2) are enzymes that compete for the same substrate, L-arginine. When NOS2 utilizes L-arginine, nitric oxide (NO) and citrulline are produced, suppressing tumor growth. On the other hand, when ASE utilizes L-arginine, ornithine and polyamines are produced, promoting tumor growth. Previous data from Dr. Zea's lab showed that cell proliferation in murine cell lines of RCC correlates with high levels of ASE expression and increased levels of NOS2 after IFNγ treatment. His lab also found that human RCC do not express NOS2 protein although the gene for this enzyme was present (Figure 1).

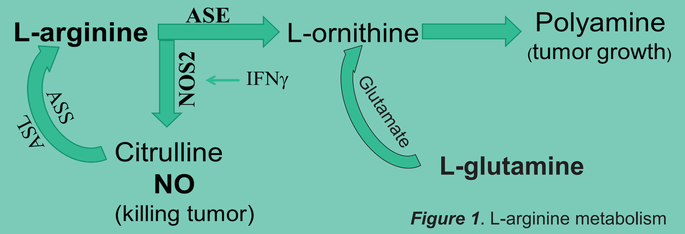

Figure 1. L-arginine metabolism

L-arginine is the only endogenous nitrogen-containing substrate of NO synthase (NOS2), and it controls the production of NO in many tumors. NOS2 enzyme is upgraded regularly by inflammatory mediators, which produces NO as long as the molecule is intact and its substrate L-arginine is available. NOS2-expressing cells can produce large amounts of NO at varying time periods. The high activity of NOS2 enzyme makes has an effective cellular defense mechanism against microorganisms. NOS2 enzyme can possibly lead to toxic levels of NO production to kill tumors. We anticipate that ASE activity could represent a very important biological marker that can be used to determine the fate of RCC development. The decreased availability of L-arginine blocks the induction of NO production in human RCC, allowing inhibition of NOS2 (caused by translation of NOS2 mRNA by mechanisms). The answer to why this happens is still a mystery and requires further investigation. Since L-arginine is the limiting factor for NOS2 protein expression, we wanted to test whether increasing L-arginine levels we were capable to demonstrate the induction of NOS2 protein and determine its function(s) by testing nitrites production. These results could provide an explanation for the lack of NOS2 protein expression and to further define a distinct mechanism by which L-arginine can regulate the activity of its associated enzyme. These findings could also have a significant impact in the biology of RCC with medical, translational implications on the treatment and/or prevention of this disease.

Objective

Our *hypothesis* is that: "Extracellular deprivation of L-arginine or intracellular arginase overexpression leads to a decreased intracellular arginine and decreased NOS2 expression". We wanted to test in human cell lines of RCC the role of L-arginine availability in the expression of NOS2 protein. Those cells should become sensitive to treatment with IFNγ.

Results

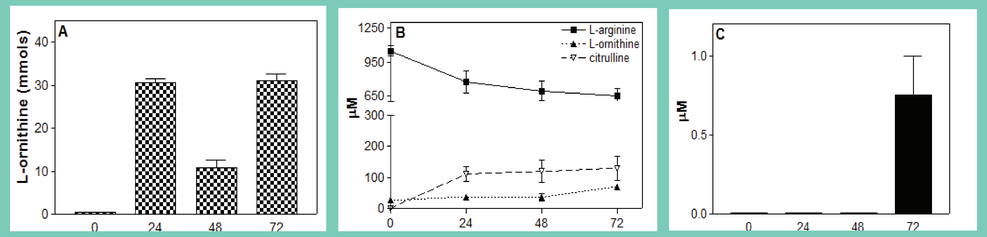

Figure 2. Base line levels for (A) arginase activity, (B) amino acid consumption and (C) nitrites in human RCC 786-0 cell line at different time points. The cells were cultured in six well plate at concentration of 300,000 cell per well. The cell line were cultured in regular media containing 1,040 µM of L-arginine and 10% fetal bovine serum. Each value represents the means of 3 different experiments ± SD.

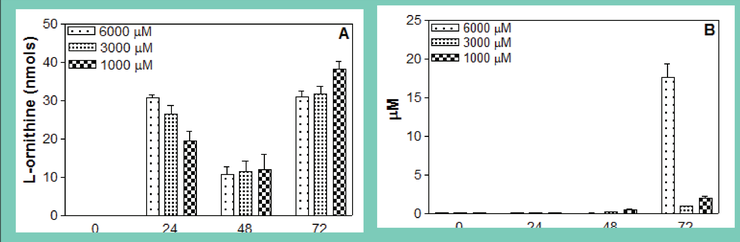

Figure 3. (A) arginase activity and (B) nitrite levels in human RCC cells cultured at different concentrations of L-arginine and different time points. Nitrite levels become apparent after 72 h in media containing 6000 µM of L-arginine. The data is representative of 2 different experiments ± SD

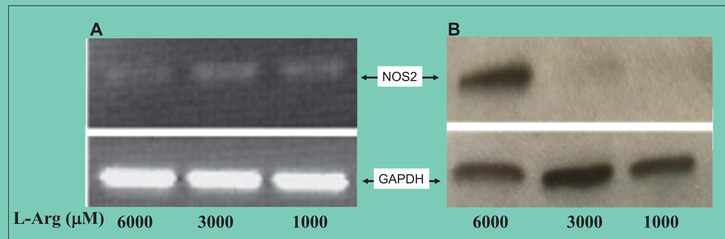

Figure 4. NOS2 gene is slightly expressed (A). No differences were found when the cells were cultured at different concentrations of L-arginine. NOS2 protein expression (B) was induced at 6000 µM of L-arginine that decreased to undetectable levels at lower concentrations of L-arginine. Gene expression was done by RT-PCR and protein by Western blot.

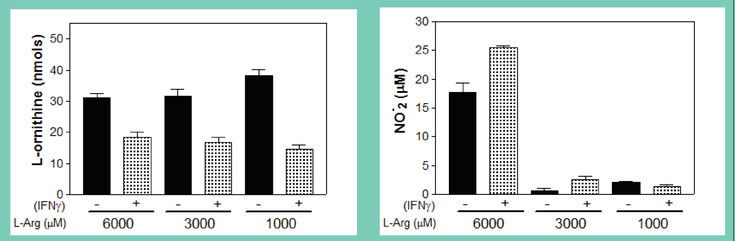

Figure 5. Cells were treated with 200 U/ML of IFNγ. (A) The levels of arginase do not show major changes at the different L-arginine concentrations. However, arginase activity decreased with IFNγ treatment in which no major differences occurred at different concentrations of L-arginine. (B) High concentrations of L-arginine (6000 µM) increased nitrite levels in untreated cells that significantly increased when treated with IFNγ.

Methodology

Three different human RCC cell lines (786-0, SKRC 28 & 45) were used for the experiments. Because of its biological properties cell line 786-0 was used throughout the experiments. The cell line was culture at different concentrations of L-arginine (6000, 3000 and 1000 µM) at different time points ranging from 24 to 124 h. Arginase, NOS2 expression, nitrite levels, amino acids and gene expression, were tested at each time point by enzymatic activity, Western blot and HPLC and RT-PCR. In addition cells were treated with 200 U/ML of human recombinant IFNγ to assess NO production and cell death.

Conclusions

1. Our hypothesis was proven correct that when arginase is overexpressed or highly produced, the levels of L-arginine are depleted in its metabolic pathway.
2. Since NOS2 is not expressed in some human RCC cell lines, it suggest, that the depletion of L-arginine regulates NOS2 expression. The addition of high L-arginine levels, significantly increased NOS2 protein expression that was enhanced when the cells were treated with IFNγ.
3. The results indicate that NOS2 transcription is activated and the uptake of L-arginine modulates the translation machinery then enhancing the levels of NOS2 expression. These effects have been seen after 72 h.
4. Our future plans contemplate the study of the mechanisms by which the inhibition of translation of NOS2 mRNA occurs. The analysis of intracellular pools of L-arginine needs also to be further investigated.
5. Other amino acids such as L-glutamine can play a determinant role in the modulation of L-arginine levels that can interfere with tumor growth-inhibition in RCC.

Acknowledgements

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